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A RESEARCH STUDY OF PHARMACEUTICAL AND ANALYTICAL STUDIES ON THE MEDICINAL HERB- KOKILAKSHA KSHAR (ASTERACANTHA LONGIFOLIA (L.) NEES)

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ABSTRACT

Kokilaksha consists of dried whole plant of *Asteracantha longifolia (L.) Nees* Syn. Hygrophila spinosa T. Anders (Fam Acanthaceae); is a spiny, stout, annual herb, common in waterlogged places throughout the country. API mentioned *kokilaksha* is *balya, Mutral, ruchya, Vajikaran*. Pharmacogenetic studies ensure plant identity and lay down standardization parameters that will help and prevents adulterations. Such studies will help in authentication of the plants and ensures the reproducible quality of herbal products which will lead to the safety and efficacy of natural products. It is a simple and reliable tool, that helps to obtain information about the biochemical and physical properties of the crude drug. In this review, the pharmacological studies conducted on *Asteracantha longifolia (L.)* Nees indicate the medicinal properties as well as the immense potential of this plant in various diseases- kidney disorder, inflammatory, liver disorder, etc. This paper explains the pharmaceutical and analytical study of the medicinal herb- *Asteracantha longifolia (L.) Nees*

Keywords: *Asteracantha longifolia, Balya, Mutral, ruchya, Vajikaran, Kokilaksha*

1. INTRODUCTION

A stout herb growing in wet places; stems numerous, fasciculate, spinous at the nodes. Leaves narrow, lanceolate, in whorls of 6, the 2 outer leaves of the whorl the larger. Flowers in sessile axillary verticals surrounded by rigid spines, bracts leaf-like; bracteoles linear-lanceolate. calyx4-partiteto the base or nearly so, the upper sepal the broader. Corolla deeply bilabiabte, the upper lip 2 lobed, the lower 3 lobed. Stamens 4, didynamous; anthers 2-celled, equal, the cells parallel, glabrous. Ovary 2-celled; ovules 4in each cell; stigma simple, acuminate. Capsule 2-celled, compressed,4-8-seeded at the base. seeds hygroscopically white -hairy. Kshar is the ashes of herbal drugs or derivatives of such ashes in the form of solutions or crystals. All of which have the basic quality of being alkaline. The kshar is the best panacea for ascites and urinary stones. Kokilaksha whole plant, seeds, and kshar are used for traditional medicinal purposes in sexual disorders like premature ejaculation, nocturnal emissions, urinary disorders like renal stones, urinary incontinence, and as an anti-inflammatory in rheumatoid arthritis and gout. Also, the leaves of this plant help to promote bile secretions and stimulates the liver, hence beneficial in hepatitis and liver diseases.

2. Vernacular names of *kokilaksha*

Sanskrit	Ikshura
	Kuliyakhara
Gujarati	Ekharo
Hindi	Talmakhana
Malyalam	Nirmuli
Marathi	Talimakhana
Tamil	Golmidi
Urdu	Talmkhana
English	Hygrophila

3. Ayurvedic properties of Kokilaksha in different *Nighantus*

S.no	Property	B.P. N	K.D. N	A. D. N	M.A. N
1.	Rasa	Madhura, amla, tikta	Madhur, amla, tikta	Madhura, tikta	Madhura, amla, tikta
2.	Guna	Pichila	Snighdha, pichila	-	Pichila
3.	Veerya	Sheeta	Sheeta	Sheeta	Sheeta
4.	Vipaka	-	-	Madhura	-

4. Chemical constituents:

a) Phytochemical studies have shown that the different parts of the Asteracantha longifolia (L.) Nees have different chemical constituents. They are as follows: The whole plant contains Lupeol, stigmasterol, isoflavone, glycoside, alkaloid, and small quantities of uncharacterized bases.

b) The seed contains- Asterol 1,2,3,4, Astercanthine, aminoacids- histidine, lysine, and phenylalanine.

c) The fresh flower contains- Apigenin-7-0-glucoside.

5. Ethnobotanical uses: The ash prepared by burning the dried plant (kshar) is given with decoction of Gokshur to treat renal calculi. The decoction of the root is used to treat jaundice and swelling of the body. Powder of seed with milk is given to treat the impotency, fewer sperm counts, and general debility.

6. Geographic distribution

The herb is distributed all over India, Sri Lanka, and Nepal especially found near moist and marshy places and unused land patches.

a. Method of preparation:

The whole plant of kokilaksha was collected and washed with water, cut into small pieces, dried well, and cleaned to remove waste material. These pieces were put in an iron vessel and burnt into ash. The ash could cool and be filtered through a sieve. Distilled water was added to the ash in the ratio of 6;1 and stirred well and allowed to stand undisturbed for 24 hrs. The next day, the supernatant liquid was decanted out and strained through a clean piece of cloth 21 times successively to get a clear liquid. The liquid Ksharodaka was then taken in a glass vessel and heated over a mild fire till the water evaporated completely. The residue obtained known as kshara was then collected by scratching the surface of a glass vessel with a knife and stored in a glass bottle.

7. Analytical study:

Pharmacognosy deals with the study of medicinal plants. It includes various studies which include the identification of drug as well as its phytoconstituents, to find the stability of the drug and standardization of the drugs. Through this study detection of adulterants can also be done. Pharmacogenetic studies ensure plant identity and lay down standardization parameters that will help and prevents adulterations. Such studies will help in authentication of the plants and ensures the reproducible quality of herbal products which will lead to the safety and efficacy of natural products. It is a simple and reliable tool, that helps to obtain information about the biochemical and physical properties of the crude drug. Steps involved in pharmacognosy:

a. Organoleptic study
b. Microscopic study
c. Physicochemical study
d. Phytochemical study

8. Organoleptic study:

Character	Leaf of Astercantha longifolia	The root of Astercantha longifolia	Flower of Astercantha longifolia
Shape	Lanceolate	Mostly adventitious	Linear lanceolate
Size	1-7cm long,0.5-1cm wide	-	2.5cm
Surface	Hairy	Rough	Hairy
Odor	No odor	No odor	-
Colour	Greenish brown	Brownish	Yellowish-brown
Taste	-	No taste	-
Fracture	Smooth	Hard	Smooth

9. Macroscopic characters of the whole plant (kokilaksha)

a. Root - Mostly adventitious, whitish to brown; no characteristic odour and taste.

b. Stem - Usually unbranched, fasciculate, sub-quadrangular, swollen at nodes, covered with long hairs which are numerous at the nodes, externally greyish brown, creamish -brown in cut surfaces, no characteristic odour and taste.

c. Leaf - Greenish-brown, 1-7 cm long, 0.5-1 cm wide, subsessile, lanceolate, acute, entire, and hairy.

d. Flower - Yellowish-brown, usually occurring in apparent whorls of eight (in 4 pairs) at each node; bracts about 2.5 cm long, with long white hairs; calyx 4-partite, upper sepal 1.6-2 cm long, broader than the other three, which are 1.3 cm long, all linear-lanceolate, coarsely hairy on the back and with hyaline ciliate margins; corolla 3.2 cm long, widely 2 lipped, tube 1.6 cm long, abruptly swollen at the top; stamens 4, didynamous, second pair larger; filament quite glabrous; anthers two-celled, subequal, glabrous; ovary two-celled with 4 ovules in each cell; style filiform, pubescent; stigma simple, involute with a fissure on the upper side.

e. Fruit - Two celled, linear-oblong, compressed, capsule about 0.8 cm long, pointed, 4- seeded.

f. Seed - Ovate, flat, or compressed, truncate at the base, 0.2-0.25 cm long and 0.1 - 0.15 cm wide, hairy but appearing smooth; when soaked in water immediately get coated with mucilage, light brown; taste slightly bitter and odour not distinct.

g. Microscopic Root

Root shows a single-layered epidermis of thin-walled, rectangular to cubical, parenchymatous cells having unicellular hairs; secondary cortex composed of round to oval or oblong, thin-walled cells having large intercellular spaces; most of these cells divided longitudinally and transversely with walls forming 4-6 or more chambers; size of these cells and intercellular spaces gradually reduce towards the inner region, where these cells are mostly radially elongated, arranged in radial rows, a few thick-walled cells found scattered singly throughout secondary cortex; secondary phloem narrow consisting of small, thin-walled, polygonal cells; phloem fibres thick-walled, occur in groups of 2-6 or singles, scattered throughout the phloem region; secondary xylem forms continuous ring; vessels angular, broader towards centre, arranged radially having spiral thickenings, surrounded by thick-walled parenchyma and xylem fibres; fibre walls uniformly thickened; multi and uniseriate medullary rays occur from primary xylem region up to secondary cortex; ray cells thin-walled, radially elongated in xylem region, circular to transversely elongated in phloem region.

h. Stem- shows somewhat sub-quadrangular outline; cork consists of 5-10 rows of rectangular, radially arranged, moderately thick-walled, brownish cells; collenchyma 4-8 layered consisting of isodiametric cells; a few thick-walled, isolated cells found scattered in this zone; cortical cells thin-walled, round, oblong, variable in size, with a number of large air cavities; a special feature of these cells is the formation of tangential and radial walls within the cell dividing it into 4-5 or more parts; most of cells contain numerous acicular crystals of calcium oxalate; endodermis single layered, composed of transversely elongate, thin-walled cells; phloem narrow, consisting of round to polygonal cells, peripheral ones larger, inner cells smaller; fibres thick-walled, single or in groups of 2-3, some cells contain calcium oxalate crystals similar to those found in cortical cells; xylem present in a ring; vessels with spiral thickenings, arranged radially; fibres elongated with wide lumen and pointed tips, medullary rays uni to multi seriate extend up to secondary cortex; ray cells thin-walled, radially elongated in secondary xylem, transversely elongated in secondary phloem; pith large, composed of polygonal, thin-walled parenchymatous cells, having small intercellular spaces; a few cells contain calcium oxalate crystals similar to those found in secondary cortex.

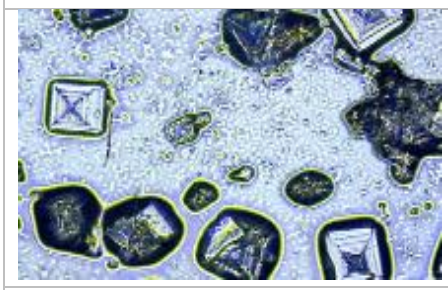
i. Leaf midrib shows concavo-convex outline; epidermis on either surface covered with thick cuticle; collenchyma 2-5 layered; stele composed of small strands of xylem and phloem having some groups of fiber; rest of tissues composed of thin-walled, parenchymatous cells, a few of them containing acicular crystals of calcium oxalate; cystolith present beneath upper and above the lower epidermal cells. Lamina - Shows epidermis single-layered on either surface, composed of thin-walled, parenchymatous, tangentially elongated cells, covered with thick cuticle; stomata diacytic, 1-5 celled hairs present on both surfaces; palisade 1-2 layered; spongy parenchyma composed of 3-5 layered, loosely arranged cells traversed by several veins; palisade ratio 6.25-15.75; stomatal index 17.24-30.78; vein islet number 17-42.

j. Fruit - Shows single-layered epidermis covered with striated cuticle followed by 5-10 layered, thick-walled, oval to hexagonal, lignified, sclerenchymatous cells.

k. Seed - Shows hairy testa composed of thin-walled, tangentially elongated cells covered with pigmented cuticle; embryo composed of oval to polygonal, thin-walled, parenchymatous cells containing oil globules.

l. Powder - Light brown; shows aseptate, elongated fibers; vessels with simple pits and spiral thickening; palisade, acicular crystals of calcium oxalate, unicellular hairs, and globules.

10. Powder Microscopy

ALK	
	
Starch grain	**Needle shaped calcium oxalate crystals**

11. Physiochemical Analysis

In physicochemical analysis, the following results have been seen-

S. No.	Test	Value
		ALK
1	Loss on Drying (%)	6.95
2	Foreign Matter (%)	0.00
3	Aqueous Extractive Value (%)	7.23
4	Alcoholic Extractive Value (%)	5.93
5	Total Ash (%)	78.43
6	Acid Insoluble Ash (%)	67.75
7	Water Soluble Ash (%)	12.38
8	Ph	9.16

12. Phytochemical screening

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. A plant cell produces two types of metabolites: primary metabolites involved directly in growth and metabolism (carbohydrates, lipids, and proteins), and secondary metabolites not involved in metabolic activity (alkaloids, phenolics, sterols, etc) but act as defence chemicals.

Freshly prepared extracts were tested for the presence of various active Phyto compounds like phenols, tannin, flavonoid, protein, reducing sugar, carbohydrates, lipids, saponin, triterpenoid alkaloid, resins, volatile oils, by the method of Kokate and Khandelwal.

Qualitative analysis of extracts to evaluate general phytochemical profile: The extracts obtained from the research drug were subject to qualitative examination as per the Pharmacopoeia of India (IP).

12.1.1. Tests for Carbohydrates

12.1.1.1. Molisch's test: 2 ml of test solution was taken in a test tube and 2 ml of the Molisch's reagent was added and shaken carefully and then about 1ml. Of conc. H_2SO_4 is poured from the side of the test tube and allowed to stand for one 1 minute. A Purple colour ring at the junction of the two layers if formed indicated the presence of carbohydrates.

12.1.1.2. Benedict's test: It is used for reducing sugars and is composed of mainly Copper sulfate and sodium hydroxide. To the 4 ml of an aqueous solution of the drug, 1 ml of Benedict's solution was added and heated almost to boiling. Formation of green, yellow, orange, red, or brown colour in order of increasing concentrations of simple sugar in the test solution, due to formation of cuprous oxide.

12.1.1.3. Barfoed's test: The test sample was dissolved in water and heated with a little of the Barfoed's reagent. The formation of a red precipitate of cuprous oxide within two minutes indicates the presence of monosaccharides.

12.1.1.4. Fehling solution test: It is generally used for reducing sugars and is composed of two solutions, which are mixed in situ. Fehling solution A composed of 0.5% of copper sulphate whereas Fehling solution B is composed of Sodium Potassium Tartrate. Equal volumes of Fehling A and Fehling B solutions were mixed (1 ml each) and 2 ml of an aqueous solution of the drug was added followed by boiling for 5-10 minutes in a water bath.

12.1.2. Tests for Alkaloids

12.1.2.1. Mayer's reagent test: 2 ml of test solution was taken in a test tube to which and 2 ml of Mayer's reagent (Potassium Mercury Iodide solution) was added. A White or Pale-Yellow precipitate if formed indicated the presence of Alkaloids except with Alkaloids of the Purine groups and a few others.

12.1.2.2. Dragon Droff's reagent test: 2 ml of test solution was taken in a test tube in which 2 ml of the Dragon Droff's reagent (Mixture of Potassium Iodide and Bismuth subnitrate solution) was added. An orange precipitate is formed indicating the presence of Alkaloids.

12.1.2.3. Wagner's Test: Drug solution + few drops of Wagner's reagent (dilute Iodine solution), formulation of reddish-brown precipitate

12.1.2.4. Hager's Test: A saturated aqueous solution of picric acid was employed for this test. When the test filtrate was treated with this reagent, an orange-yellow precipitate was obtained which indicates the presence of alkaloids.

12.1.3. Test for Amino acids

12.1.3.1. Ninhydrin test:

The Ninhydrin test is used to detect the presence of alpha-amino acids and proteins containing free amino groups. Protein solution when heated with ninhydrin molecules, it gives characteristic deep blue or pale-yellow colour due to the formation of a complex between two ninhydrin molecules and nitrogen of free amino acid.

12.1.3.2. Tests for Proteins

Biuret test: A few mg of the residue was taken in water and 1 ml of 4% sodium hydroxide solution was added to it, followed by a drop of 1% solution of copper sulphate. The development of violet or pink colour indicates the presence of proteins.

12.1.3.3. Xanthoprotic test: A small quantity of test sample was taken with 2 ml of water and 0.5 ml of concentrated nitric acid was added to it. The development of yellow colour indicates the presence of proteins.

12.1.3.4. Millons test: A small quantity of test sample was taken and 2 to 3 ml of millions reagent was added. The white precipitate slowly turns pink, indicating the presence of proteins.

12.1.4. Test for saponin

12.1.4.1. Foam test: A small quantity of the test sample was taken in a test tube and shaken vigorously with a small amount of sodium bicarbonate and water. A stable, characteristic honeycomb-like froth indicates the presence of saponins.

12.1.4.2. Biuret test: A few mg of the residue was taken in water and 1 ml of 4% sodium hydroxide solution was added to it, followed by a drop of 1%

solution of copper sulphate. The development of violet or pink colour indicates the presence of proteins.

12.1.4.3. Xanthoprotic test: A small quantity of test sample was taken with 2 ml of water and 0.5 ml of concentrated nitric acid was added to it. The development of yellow colour indicates the presence of proteins.

12.1.4.4. Millons test: A small quantity of test sample was taken and 2 to 3 ml of millions reagent was added. The white precipitate slowly turns pink, indicating the presence of proteins.

12.1.5. Test for saponin

12.1.5.1. Foam test:

A small quantity of the test sample was taken in a test tube and shaken vigorously with a small amount of sodium bicarbonate and water. A stable, characteristic honeycomb-like froth indicates the presence of saponins.

12.1.6. Test for glycosides

12.1.6.1. Borntragor's Test:

1 ml of Benzene and 0.5 ml of dilute ammonia solution were added to the ethanolic extract and were observed in the form of reddish-pink colour.

12.1.7. Test for Phenolic Compound

The extract was taken in water and warmed; to this 2 ml of ferric chloride solution was added and observed for the formation of green and blue colour.

12.1.8. Test for Flavonoids

12.1.8.1. Shinods test:

A small quantity of test sample was dissolved in 5 ml ethanol (95%v/v) and reacted with a few drops of concentrated hydrochloric acid and 0.5 gm of magnesium metal. The appearance of pink, crimson, or magenta colour within a minute or two indicates the presence of flavonoids.

12.1.9. Test for Steroids

12.1.9.1. Salkoweski reaction:

A few mg of extract was taken in 2 ml of chloroform and 2 ml of concentrated sulphuric acid was added from the side of the test tube. The test tube was shaken for a few minutes. The development of red colour indicates the presence of steroids.

12.1.10.Test for Tannins

12.1.10.1. Ferric chloride solution: A 5 percent solution of ferric chloride in 90 % alcohol was prepared. Few drops of this solution were added to a little of the above filtrate. The appearance of dark green or deep blue colour indicates the presence of tannins

12.1.10.2. Lead acetate: A 10 percent w/v solution of basic lead acetate in distilled water was added to the test filtrate. The development of precipitate indicates the presence of tannins.

12.1.10.3. Pot. Dichromate: A solution of potassium dichromate was added to the filtrate. The appearance of dark colour indicates the presence of tannins.

Name of Test	ALK	
	Aqueous Extract	Alcoholic Extract
Carbohydrate		
Molish test	- ve	+ ve
Benedict test	+ ve	- ve
Fehling test	- ve	- ve
Alkaloids		
Dragendorff test	- ve	- ve
Wagner's test	- ve	- ve
Hager's test	- ve	- ve
Amino acids		
Ninhydrine	- ve	+ ve
Protein		
Biuret test	- ve	- ve
Xenthoprotic test	- ve	- ve
Saponin		

Foam test	- ve	- ve
Glycosides		
Borntrager's test	- ve	- ve
Phenolic compound		
Phenolic test	- ve	- ve
Steroids		
Salkowaski	- ve	- ve
Tannins		
$Fecl_3$	- ve	- ve
Lead acetate	+ ve	+ ve
Pot. Dichromate	- ve	- ve

12. Chromatography:

Thin layer Chromatography is a tool for the separation and identification of chemical constituents. Thin-layer chromatography is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through adsorption and a mobile phase in the form of a liquid. The adsorbent is a relatively thin, uniform layer of dry finely powdered material applied to a glass, plastic, or metal sheet or plate.

13. Chromatography plates

T.L.C. plate coated with a 0.25 mm layer of silica gel 60 F_{254} with fluorescent indicator was used. (Each plate dimension is 10 cm long and 2 cm in width)

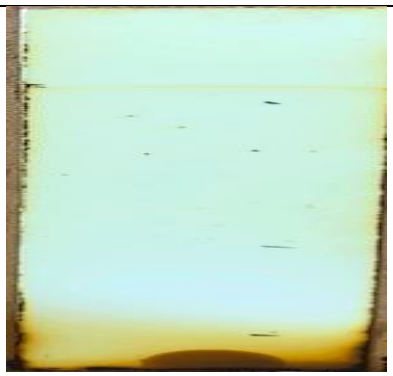
- Activation of pre-coated Silica gel 60 $F_{254:}$ Plates were dried in a hot oven at 105^0 C for one and half hours.
- Test solution: Alcoholic Extract
- Preparation of mobile solution: Toluene: Ethyl Acetate: Formic acid (6:3: 1)
- Visualization: Iodine Vapour

14. Rf Value: Measured and recorded the distance of each spot from the point of its application and calculated Rf. Value by dividing the distance traveled by the spots by the distance traveled by the front of the mobile phase.

a. Calculation of Rf Value

Distance traveled by solute from origin line R_f = Distance traveled by solvent from origin line

ALK



R_f Value: 0.09, 0.45

15. CONCLUSION

The whole plant of Asteracantha longifolia (L.) Neesis used in traditional ayurvedic medicine for the treatment of sexual disorders, inflammatory disorders, and urinary disorders. Asteracantha longifolia (L.) Neesmainly contains lupeol, stigmasterol, an isoflavone glycoside, an alkaloid, and small quantities of uncharacterized bases. This paper explains the evidence-based information regarding the pharmacological activity of this plant. Every plant is pharmacologically important. The Pharmacognostical values such as total cash value, water-soluble and insoluble ash value along with fluorescence values, micro, and macroscopic values are stated. Phytochemical investigation studies reveal that all the plants contain a lot of phytochemicals such as alkaloids, steroids, flavonoids, tannins, carbohydrates, and amino acids. In the changing global scenario, the interest in plants with medicinal value is increasing substantially in the primary healthcare system both in developed and developing countries. therefore, the information will help the scientists and researchers to screen the compounds responsible for different bioactivities, and elucidate the molecular mechanism of action.

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